

# maternal vitamin D intake

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## **Maternal vitamin D intake and serum 25-hydroxyvitamin D (25(OH)D) levels associated with blood pressure: A cross-sectional study in Padang, West Sumatra**

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### **ABSTRACT**

**Introduction:** The association between vitamin D inadequacy and blood pressure (BP) has been studied in several populations. We examined the association of maternal vitamin D intake and serum 25(OH)D levels and BP among pregnant women in West Sumatra. **Methods:** This study was conducted using a comparative cross-sectional study in a maternal clinic selected by convenience in Padang. Pregnant mothers who attended the clinic in July-August 2015 were recruited. Inclusion criteria were pregnancy between 28-42 weeks, aged 20-35 years, and with less than three parity status. A total of 56 women were recruited and divided equally into either normotension or hypertension groups. Subjects with blood pressure less than 120 mmHg (SBP) and 80 mmHg (DBP) were placed in the normotension group, while subjects with  $\geq 140$  mmHg (SBP) and/or  $\geq 90$  mmHg were in the hypertension group. Subjects completed a pre-tested semi-quantitative food frequency questionnaire. Three ml of non-fasting blood was drawn from each subject for determination of 25(OH)D, urea, creatinine, leukocyte, and blood glucose levels. **Results:** There was a significant difference in mean serum 25(OH)D, between the normotension and hypertension groups, at  $36.85 \pm 21.58$  pg/ml and  $17.36 \pm 7.91$  pg/ml, respectively. Only 20% of participants from the hypertension group had adequate vitamin D intake. Blood pressure status had a significant association with vitamin D intake status ( $p=0.001$ ). **Conclusion:** Maternal vitamin D intake and 25(OH)D levels were associated with blood pressure status in this sample population. Further studies with a larger sample population are suggested to verify the findings of this study.

**Keywords:** Blood pressure, pregnancy, 25(OH)D, vitamin D intake

### **INTRODUCTION**

One of the most common medical problem encountered during pregnancy is hypertension, which complicates 10% of all pregnancies (Berhan, 2016).

Hypertensive disorders of pregnancy (HDP) include chronic hypertension (occurring before 20 weeks gestation, or persisting longer than 12 weeks after delivery), gestational hypertension

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(occurring after 20 weeks gestation), pre-eclampsia (occurring after 20 weeks gestation and either proteinuria or thrombocytopenia), and eclampsia (pre-eclampsia followed seizure) (Leeman *et al.*, 2016). In addition, more than 8% of all pregnancies are related to cardiovascular death as a result of hypertensive disorders during pregnancy (HDP) (Naderi *et al.*, 2017). Hypertension may also increase the risk of adverse maternal and foetal outcomes, such as preterm birth, intrauterine growth restriction (IUGR), perinatal death, acute renal or hepatic failure, antepartum haemorrhage, postpartum haemorrhage, low birth weight and maternal death (Kendrick *et al.*, 2015; Seyom *et al.*, 2015).

The United Nations Children's Fund (UNICEF) has found that women living in developing countries of the world are 300 times more likely to die because of childbirth or pregnancy-related complications than women living in developed countries. Maternal mortality is the global health indicator with the largest disparity between developed and developing countries. Every year, more than half a million women die because of pregnancy or childbirth complications.

The mean maternal mortality ratio (MMR) in Indonesia fell from 385 to 216 deaths per 100,000 live births in 1990-2015. Despite the 44% reduction, this condition is still far from the recommendation of Millennium Development Goal (MDG), which must reach 75%. Sustainable Development Goal (SDG) is targeting maternal deaths to be less than 70 per 100 000 live births in 2030. One of the main causes of maternal death is gestational hypertension (Alkema *et al.*, 2016; Statistics Indonesia *et al.*, 2013).

Vitamin D plays a critical role in regulating the Renin-Angiotensin System (RAS) and vascular smooth muscle. Thus, vitamin D has the

potential to affect blood pressure (Wong *et al.*, 2010). Ullah *et al.* (2009) reported that vitamin D deficiency could increase the risk of hypertension by as much as 67%. The effect of lifestyle, inadequate maternal vitamin D intake, and lack of exposure to sunlight may lead to low vitamin D status (Ullah *et al.*, 2009). Li *et al.* (2004) had reported that low serum 25(OH)D levels could enhance Renin-Angiotensinogen II production, causing elevated blood pressure.

Low levels of 25(OH)D during the third trimester may lead to increased blood pressure or hypertension. Liu *et al.* (2013) studied low vitamin D status in pregnant mice leading to symptoms of pre-eclampsia that influenced Renin-Angiotensinogen II receptor. This condition affects the flow of blood, causing constrictions that may lead to increased blood pressure during pregnancy (Liu *et al.*, 2013). This study aimed to assess the association between maternal vitamin D intake and 25(OH)D serum levels with blood pressure status in pregnant subjects.

## MATERIALS AND METHODS

### Study design and subjects

This study was a comparative cross-sectional study with 61 pregnant mothers recruited from a maternal clinic selected by convenience in Padang, Indonesia. Pregnant mothers with a history of diabetes mellitus, kidney disorder, pre-eclampsia, eclampsia, anaemia, multiple pregnancies, and those taking medications that might affect vitamin D metabolism were excluded. This study was conducted from July-August 2015.

The subjects were healthy pregnant mothers with hypertension. The inclusion criteria were pregnant mothers within 28-42 weeks of gestation, aged 20-35 years, and with less than three parity statuses. All subjects were informed of the purpose of the study

and requested to participate after giving written consent; five subjects opted to drop out at this point. The subjects were divided equally into two groups namely, subjects with normotension ( $n=28$ ) and those with hypertension ( $n=28$ ).

#### Demographic and anthropometry data

Age, parity status, actual maternal body weight at third trimester, and height were recorded when the pregnant mothers attended antenatal care (ANC) in the selected maternal clinic. Unfortunately, we were not able to get pre-pregnancy body weight data. Body Mass Index (BMI) was presented in  $\text{kg}/\text{m}^2$ .

#### Dietary assessment

Maternal vitamin D intake was assessed by using a semi-quantitative food frequency questionnaire (SQ-FFQ) that was developed and validated by Lipoeto *et al.* (2004). The SQ-FFQ had been adapted to food habits of Minangkabau, an ethnic group inhabiting the Highlands of West Sumatra. The SQ-FFQ consisted of 223 general food items including potential sources of vitamin D in the targeted population.

Energy and nutrient intake were determined using the SQ-FFQ, which provided a snapshot of the dietary intake among the Minangkabau ethnic group residing in Padang. The SQ-FFQ also provided detailed information of the food choices and sources of nutrients consumed by the subjects in the last three months. Potential vitamin D-rich foods in the area were added to the questionnaire. The Bowes & Chur's Food Values of Portions and the U.S. Department of Agriculture (USDA) Foods database (Pennington & Spungen, 2010; USDA, 2015) were used to estimate vitamin D content in the foods consumed. Intakes of energy, macronutrients (carbohydrate, protein, fat) and micronutrients were obtained using the Indonesian Food Database

and Nutrisurvey (Version 2007, SEAMEO-TROPMED RCCN University of Indonesia, Jakarta, Indonesia).

Subjects with a daily energy intake of  $<500$  kcal or  $>3500$  kcal were excluded from further data analyses of food or nutrient intake because extreme values could interfere with the results (Willett, 1990). Nutrient intake was expressed in actual grams/day for macronutrients,  $\mu\text{g}/\text{day}$  for maternal vitamin D intake and as a percentage of total energy intakes. Maternal vitamin D intake status was defined as  $<15$   $\mu\text{g}/\text{day}$  for inadequate and  $\geq 15$   $\mu\text{g}/\text{day}$  for adequate.

#### Vitamin D blood samples

Non-fasting venous blood samples (3ml) were collected from each pregnant mother in the third trimester of pregnancy. The blood samples were centrifuged at 3500 rpm for 10 min at  $4^{\circ}\text{C}$  (Eppendorf Centrifuge 5810R, Hamburg, Germany). The serums were placed in aliquot vials and stored at  $-20^{\circ}\text{C}$  to  $70^{\circ}\text{C}$  until analysed. Serum samples were protected from light oxidation and thawed once before analysis, which was completed after 6 months of data collection.

The blood samples were centrifuged at Dr M. Djamil Hospital, Padang and brought to Biomedical Laboratory of The Medical Faculty of Andalas University for Enzyme-linked Immunosorbent Assay (ELISA). The 25(OH)D for human reagent was checked by E-EL-0016 DVD/DHVD3 (1,25-Dihydroxyvitamin D<sub>3</sub>), using ELISA kit (Elabscience Biotechnology Inc., Wuhan, Hubei, China) utilising the Sandwich-ELISA method.

The micro ELISA plate provided in this kit had been pre-coated with an antibody specific to 25(OH)D. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution whose colour turned to yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of  $450\pm 2$  nm. The OD value

is proportional to the concentration of 25(OH)D. The serum 25(OH)D results were expressed in pg/ml. The lower and upper limits of detection were 2.0 and 200.0 pg/ml, respectively. In the third trimester of pregnant mothers, the recommended level for 25(OH)D levels was 60-119 pg/ml (Abbassi-Ghanavati et al., 2009).

#### Blood pressure measurement

Two measurements of general blood pressure were taken within a 5-minute period with a random zero sphygmomanometer with subjects in a sitting position with their backs supported and their legs uncrossed. Measurements were taken by trained midwives on-site during antenatal care check-up. The definition of hypertension as defined by the American College of Cardiology (ACC) and the American Heart Association (AHA), which had released a clinical practice guideline for the prevention, detection, evaluation, and treatment of high blood pressure (BP) in adults (Carey et al., 2018) was used.

This guideline defines hypertension as a systolic blood pressure (SBP) equal to or greater than 140 mmHg or a diastolic blood pressure (DBP) equal to or greater than 90 mmHg. Pregnant mothers with blood pressure less than 120 mmHg (SBP) and 80 mmHg (DBP) were placed in the normotension group, 120-139 mmHg (SBP) or 80-89 mmHg (DBP) were in the prehypertension group, and with either  $\geq 140$  mmHg (SBP) and/or  $\geq 90$  mmHg) were in the hypertension group.

#### Statistical analysis

All data analyses were performed by statistical software package SPSS 20 and were expressed by mean (SD), and as frequencies and percentages for categorical variables. The presence of a normal distribution for each

variable was tested by the Shapiro Wilk test. The subjects were stratified by hypertension and normotension group, and by maternal vitamin D intake status as defined previously. Comparisons between groups were performed using a chi-square test for categorical variables. Continuous variables were compared between groups by independent samples t-tests for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. Pearson chi-square, Yates corrected chi-squared, and Fisher's exact tests were performed for categorical variables. The student's t-test was used to compare the differences of mean of 25(OH)D serum levels between blood pressure classification. Finding at  $p < 0.05$  for a two-sided test was considered statistically significant with 95% Confidence Interval (CI) to see the relationship.

#### Ethics approval

The Research Ethics Committee of Medical Faculty, Andalas University, West Sumatera, Indonesia approved the study protocol. The letter number is 347/KEP/2014.

#### RESULTS

A total of 56 pregnant mothers aged 21-35 years participated in this study. The characteristics of the subjects according to blood pressure status are shown in Table 1. The association between maternal characteristics and blood pressure status showed variable results. There was no significant difference between parity status, age groups, and BMI status and blood pressure status.

Out of the 56 subjects, only twenty (35.7%) had adequate vitamin D intake of more than  $15 \mu\text{g/day}$ . There was a significant difference in vitamin D intake between normotension and hypertension subjects (Table 2).

**Table 1.** Characteristics of study participants according to blood pressure status

Characteristics	N (%)	Normotension	Hypertension			<i>p</i> -value <sup>1</sup> OR (95%CI)
Age (years)				(%)	(%)	
a. 21-25	15(26.8)	7 (46.7)	8 (53.3)			0.666
b. 26-30	25(44.6)	14 (56.0)	11 (44.0)			
c. 31-35	16 (28.6)	7 (43.8)	9 (56.2)			
Parity status						0.060
a. Nulliparous	31 (55.4)	12 (38.7)	19 (61.3)			0.35 (0.11-1.05)
b. Multiparous	25 (44.6)	16 (64.0)	9 (36.0)			
Vitamin D intake ( $\mu$ g/day)						<0.001 <sup>1</sup>
a. Adequate ( $\geq 15$ )	20 (35.7)	17 (85.0)	3 (15.0)			0.078 (0.019-0.32)
b. Inadequate (<15)	36 (64.3)	11 (30.5)	25 (69.4)			
Height T3 (cm)	56 (100.0)	28 (50.0)	28 (50.0)			0.904
BMI T3, (kg/m <sup>2</sup> )						
a. <18	0	0 (0.0)	0 (0.0)			
b. 18-25	17 (30.4)	9 (52.9)	8 (47.1)			0.685
c. 25-30	33 (58.9)	17 (51.5)	16 (48.5)			
d. $\geq 30$	6 (10.7)	2 (33.3)	4 (66.7)			
Urea (mg/dL)	56 (100.0)	28 (50.0)	28 (50.0)			0.818
Creatinine (mg/dL)	56 (100.0)	28 (50.0)	28 (50.0)			0.283
Blood glucose (mg/dL)	56 (100.0)	28 (50.0)	28 (50.0)			0.528
Leucocyte (mm <sup>3</sup> )	56 (100.0)	28 (50.0)	28 (50.0)			0.069

SD=Standard deviation; BMI=Body mass index; T3=Third trimester.

<sup>1</sup>Categorical variables were expressed as numbers and percentages, and analysed using a chi-square test.

<sup>2</sup>Significant at  $p < 0.01$  level.

The difference in serum 25(OH)D levels between hypertension and normotension pregnant mothers was significant. Table 3 shows that the mean serum 25(OH)D level in normotension pregnant mothers was  $6.85 \pm 21.58$  pg/ml, while that of hypertension pregnant mothers was  $17.36 \pm 7.91$  pg/ml. The minimum and maximum values of 25(OH)D for all subjects was 5.72 pg/ml

and 93.92 pg/ml respectively.

The mean systolic blood pressure of pregnant mothers was  $108.29 \pm 10.21$  mmHg for normotension group, and  $152.14 \pm 6.23$  mmHg for hypertension group. The mean diastolic blood pressure of pregnant mothers was  $71.93 \pm 5.56$  for normotension group and  $95.36 \pm 2.61$  for hypertension group.

The mean maternal vitamin D intake

**Table 2.** Association of dietary vitamin D intake and blood pressure status

Vitamin D consumption ( $\mu$ g/day)	Blood pressure				Total		<i>p</i> -value <sup>1</sup>	OR (95%CI)
	Hypertension		Normotension		N	%		
$\leq 15$	n	%	n	%				
$\leq 15$	25	69.4	11	30.6	36	64.3		
>15	3	15.0	17	85.0	20	35.7	0.0001	0.078
Total	28	50.0	28	50.0	56	100.0		

$\mu$ g=microgram

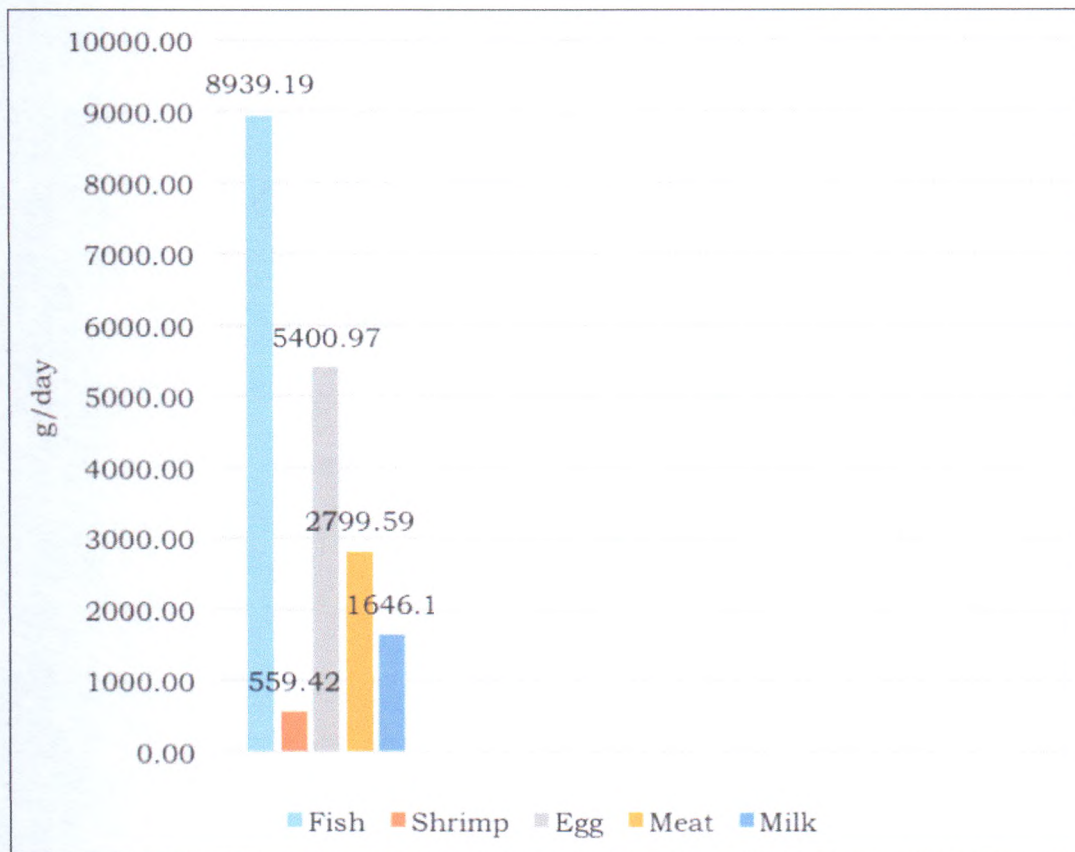
<sup>1</sup>Categorical variables were expressed as numbers and percentages, and analysed using a chi-square test. Differences were considered statistically significant at  $p < 0.05$  level.

**Table 3.** Mean of 25(OH)D serum levels, blood pressure, and maternal Vitamin D intake between normotension and hypertension pregnant mothers

Variables	Normotension		Hypertension		p-value
	Mean	SD	Mean	SD	
25(OH)D levels (pg/ml)	36.85	21.58	17.36	7.91	0.0001*
Vitamin D intake ( $\mu$ g)	16.61	9.39	10.22	7.23	0.006*
Blood pressure (mmHg)					
a. Systolic	108.29	10.21	152.14	6.23	0.002*
b. Diastolic	71.93	5.56	95.36	2.61	0.006*

SD=Standard Deviation; Mean of variables were analysed using independent sample *t*-test. Mean differences were considered statistically significant at  $p < 0.05$  level.

\*Independent sample *t*-test significant at  $p < 0.01$  level.

**Figure 1.** Sources of vitamin D-rich food (gram/day) consumed by pregnant mothers.

of normotension and hypertension was  $16.61 \pm 9.39$   $\mu$ g/day and  $10.22 \pm 7.23$   $\mu$ g/day, respectively. The minimum-maximum value of maternal vitamin D intake for all subjects was 4-36  $\mu$ g/day. Based on the independent sample *t*-test analysis, maternal vitamin D intake

was significantly different between normotension and hypertension mothers.

Vitamin D is not found naturally in foods commonly consumed by the subjects, and the highest sources of dietary vitamin D were fish, fortified

foods (mostly from milk and dairy products), and egg and meat (Figure 1).

The mean difference of dietary consumption of vitamin D-rich foods in pregnant mothers with normal blood pressure was  $834.93 \pm 242.49$  g/day, and  $385.08 \pm 145.14$  g/day for high blood pressure. Those with a higher intake of vitamin D-rich foods had lower blood pressure compared to those with lower intake of vitamin D-rich foods during pregnancy. The average intake of vitamin D sources during pregnancy in high blood pressure pregnant mothers was lower than that of normal blood pressure pregnant mothers ( $p=0.001$ ).

## DISCUSSION

Vitamin D deficiency and insufficiency are common throughout the world. Large epidemiological studies showed high prevalence of vitamin D deficiency in women, including antenatal and lactating mothers (Mithal *et al.*, 2009). This study revealed that the average maternal vitamin D intake was  $13.42 \pm 8.91$   $\mu\text{g}/\text{day}$ , with the lowest and highest intake at 4  $\mu\text{g}/\text{day}$  and 36  $\mu\text{g}/\text{day}$ , respectively

There was a significant relationship between maternal vitamin D intake and blood pressure among the third trimester pregnant mothers. A total of 64.3% pregnant mothers in their third trimester consumed less than the recommended amount of vitamin D. Hyppönen *et al.* (2005) reported a relationship between the risk of high blood pressure with vitamin D deficiency. Low maternal vitamin D status may influence the risk of pre-eclampsia because of its effect on transcriptional gene, immune function, and blood pressure. Some experts suggest that 25(OH)D plays a role in the regulation of genes that are responsible for placental invasion and blood vessel formation (Evans *et al.*, 2004). The role of vitamin D in the modulation of immune

function may affect immunologic response to the foetus.

In our previous study, we reported that 94.9% of young women aged 20-50 years had low 25(OH)D serum levels (Keumala Sari, 2013). Synthesis of vitamin D during pregnancy is higher than in non-pregnant mothers. Kidney and placenta produce vitamin D, 25(OH)D, by CYP27B1 enzyme activity. The production of 25(OH)D increases two folds in the third trimester of pregnancy. Their body's need for 25(OH)D serum leads to increased absorption of calcium in the intestine (Ma *et al.*, 2012).

During the third trimester, pregnant mothers should increase their vitamin D intake. The need for vitamin D may be greater during pregnancy because of the physiologically higher levels of 25(OH)D seen in the second and third trimester (Hollis and Wagner, 2013). The physiological increase of active metabolite vitamin D during pregnancy, due to elevated intestinal calcium absorption, raises the foetal need of calcium (250 mg/day in the third trimester), and may affect the immunomodulation system and placental development. This evidence again indicates the importance of having sufficient vitamin D intake during pregnancy (Specker, 2012).

Vitamin D supplementation is suggested for women with vitamin D deficiency, dark skin, less sun exposure, usage of long dress and veil daily, vitamin D metabolism disorders, pregnancy and breastfeeding mothers (Mithal & Kalra, 2014). Vitamin D supplementation during pregnancy is suggested at 2000-4000 IU/day for a safe dose (Hollis & Wagner, 2013).

The recent study by Bean *et al.* (2017) in the U.S. population reported that vitamin D and calcium supplementation showed a positive association with reducing blood pressure. Low levels of vitamin D may impair calcium homeostasis by the renin-angiotensin



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